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Testicular cycles of the Ringtail, *Bassariscus astutus* (Carnivora: Procyonidae)

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Abstract

Annual variation of testes in *Bassariscus astutus* was studied by palpation and morphological technique. Seasonal changes of spermatogenesis and testicular weight/size were compared. It could thus be confirmed that in Arizona the mating season extends from late winter into spring and that testes become aspermic in summer and reach their smallest size in autumn.

Introduction

The purpose of this study is to ascertain the annual cycle (seasonal development and regression) of the testes in *Bassariscus astutus*, and the determination of the male's ability (or inability) to produce active spermatozoa throughout the year. Ringtails appear to be seasonally monestrous, with the mating season extending from about mid February to May as evidenced by the majority of litters occurring during May and June (Grinnell et al. 1937; Poglayen-Neuwall and Poglayen-Neuwall 1980; Taylor 1954; Toweill 1976). There exists, to date, no histological study supporting this assumption.

Material and methods

Subjects and context

Thirteen wild-caught males were palpated at the time of capture and released while 23 captive males, ages ranging from 1 to 7 years, were palpated bi-weekly throughout the year to determine the size of the testes. In addition, 21 pairs of testes were taken from sacrificed animals, trap casualties, and fresh road kills. These were acquired for each month of the year. Ages of the animals, if not known, were estimated by tooth wear and by the morphology of the baculum (after Wood 1952). Testes were excised, and after removal of the tunica vaginalis, weighed (including epididymis) to the nearest 0.01 g. The greatest length and width (exclusive epididymis) were recorded to the nearest 0.01 mm. Each testis was hemisected longitudinally. Of each pair one testis was fixed in 10% phosphate-buffered formalin of pH 7-0, for light microscopy. The other was fixed in a combination of 4% commercial formaldehyde and 1% glutaraldehyde in a buffer of 176 m/O/sm/liter (McDowell and Trump 1976) for electron and/or light microscopy. Specimens for electron microscopy were not available from the months of January, September und October.

Histological methods

For light microscopy longitudinal slices of the formalin-fixed testicle were cut, dehydrated and embedded in paraffin. Sections were cut a 5 µm and stained with hematoxylin and eosin. For transmission electron microscopy blocks no larger than 1 µm in any dimension were cut from the most superficial tissue, postfixed in 2% OsO_4 in phosphate buffer, pH 7.4 for 1 hour, dehydrated in an ascending series of alcohol and propylene oxide and embedded in an epon-araldite mixture (Mollenhauer 1963). These sections were cut with glass knives, mounted on naked copper grids and stained with uranyl acetate and lead citrate.

Seminiferous epithelial area (SEA) (Basurto-Kuba et al. 1984) was estimated by photographing randomly selected seminiferous tubules at a magnification of ×50. Photographic prints were made to give a final magnification of ×425. Photographs were taken with a Nikon camera with Nikon attachment AMF on a Nikon labophot microscope. The basal membrane and the luminal margin were delineated to include all epithelial cells in 15 to 40 approximately round tubules in each animal. Coordinates of the seminiferous tubules were then digitized using a BQ CAM microcomputer system (R. and M. Biometrics, Inc., 5611 Ohio Ave., Nashville, TN 37209). The epithelial area of each tubule cross section was determined as the area inclosed by the basal membrane minus the area of the lumen. The SEA of each animal was expressed in square mm as the mean of the tubules measured. Student's t-test was used to test significance of differences between animals.

Five degrees of activity using a scale of 0 to 4+ were used to characterize sections of each testicle. For spermatogenesis in seminiferous tubules activity corresponded to the following: 0 = absence of sperm; 1+ = at least 1 sperm/sperm head; 2+ = several sperm/sperm heads; 3+ = moderate number of sperm/sperm heads; 4+ = many sperm/sperm heads. A similar scale was used to evaluate the number of sperm in the epididymis.

Results

Summary of the morphologic evaluation, morphometry (SEA) and statistical significance testing is indicated in Table 1. In late winter and early spring testes were most active. SEA was statistically low in December, January and February. A significant increase occurred in March, April and May and a marked decrease in June. From July to November testes were inactive. A rapid increasee in SEA between December and January was apparent.

The quantitiy of sperm in seminiferous tubules lagged 1 to 2 months behind changes in SEA (Table 1). Similarly changes in sperm concentration in epididymis usually was recorded a month later than in seminiferous tubules.

Table 1. Spermatogenetic stages

Month	SEA[1]	Sperm in sem. tub.	Sperm in epididymis
Jan.	8100	+ +	+ +
Feb.	7900	+ + +	+ +
Mar.	8500	+ + +	+ +
Apr.	4350	+ + + +	+ + +
May	4060	+ + +	+ + + +
June	4180	+	+ +
July	1900	0	0
Aug.	1680	0	0
Sept.	2560	0	0
Oct.	2040	0	0
Nov.	3190	0	0
Dec.	3250	+	0

[1] Calculated from the first day of each month.

Histopathological characteristics of the testis and epididymis are related to the stage of activity which correlated with the time of year (Figs. 1–5) and resembled those of other species in similar state of activity, e.g. Norway rat, rabbit, European boar and domestic cat (Moens et al. 1975; Morton et al. 1986; Basurto-Kuba et al. 1984; Elcock et al. 1984). Degenerative changes occurred in the seminiferous epithelium during the period of declining activity, although there were a few stem cells present.

Weights of testes (Table 2) were lowest in September-October with steep increase toward March. Testes in March show maximum weight. From April to September the weight declines steadily. Similarly, linear measurements of testes show maximum length and width in March, and minimum length and width in September-October (Fig. 6).

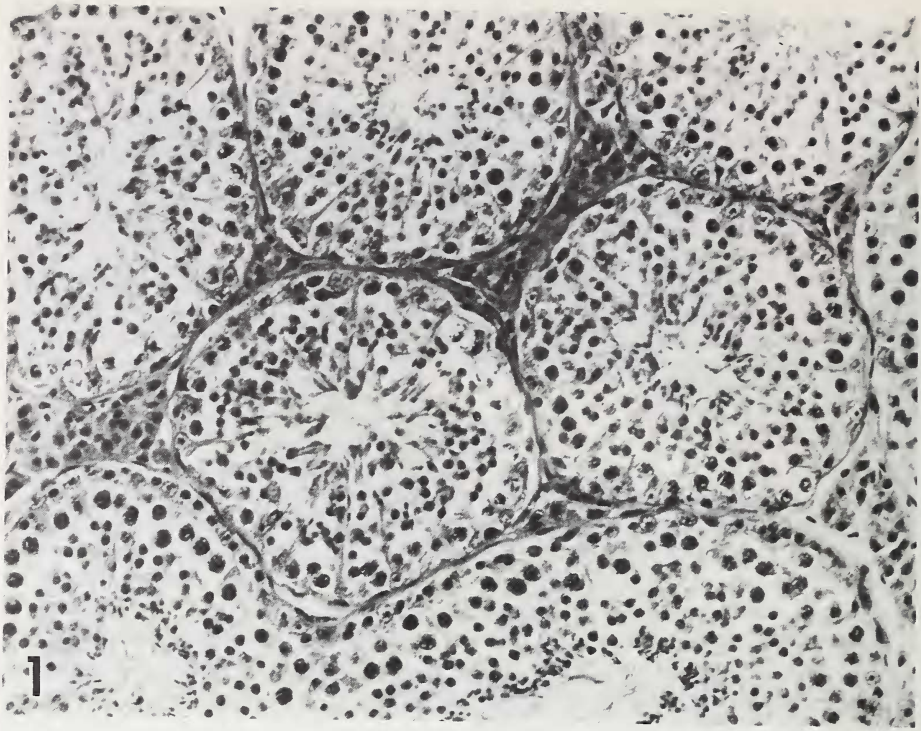

Fig. 1. Seminiferous tubules representative of late winter and early spring (January). (×200)

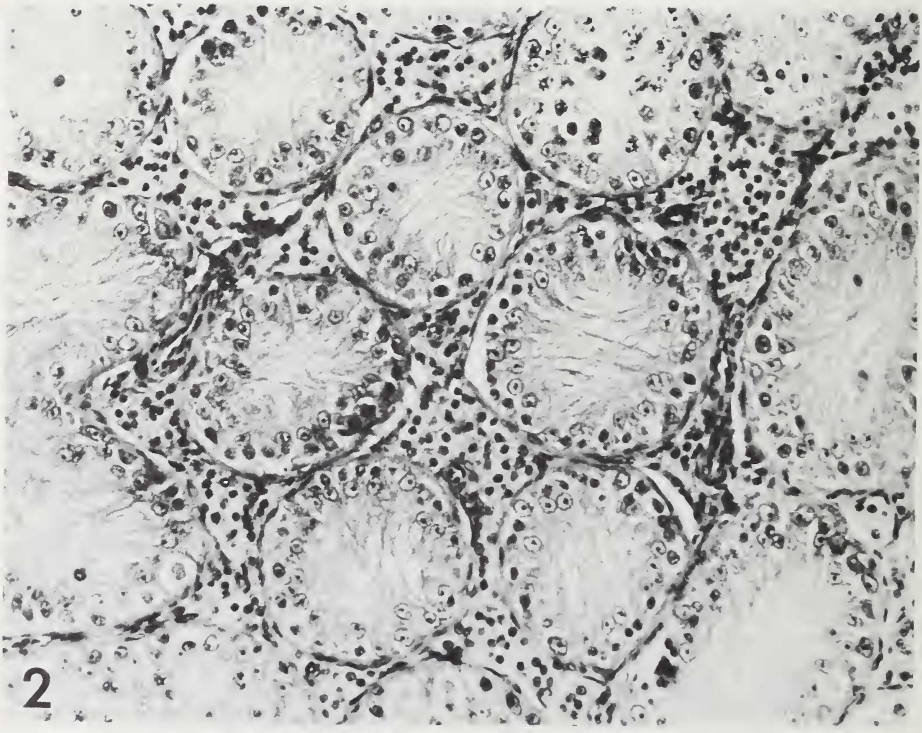

Fig. 2. Seminiferous tubules of inactive phase. Sertoli cells predominate (September). (×200)

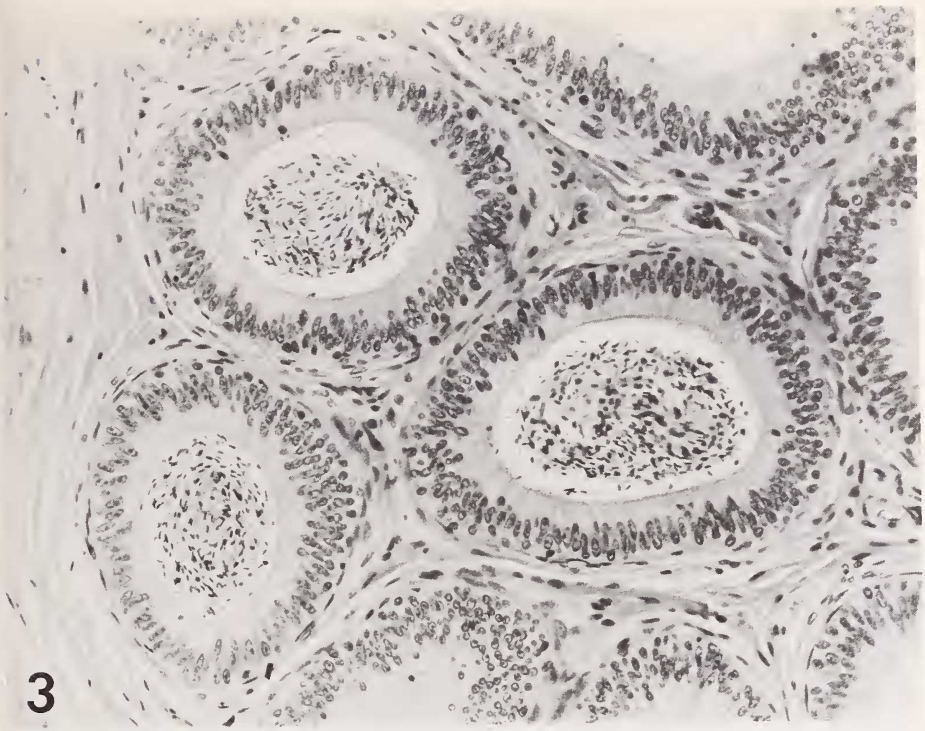

Fig. 3. Epididymis from most active phase of production (January). (×200)

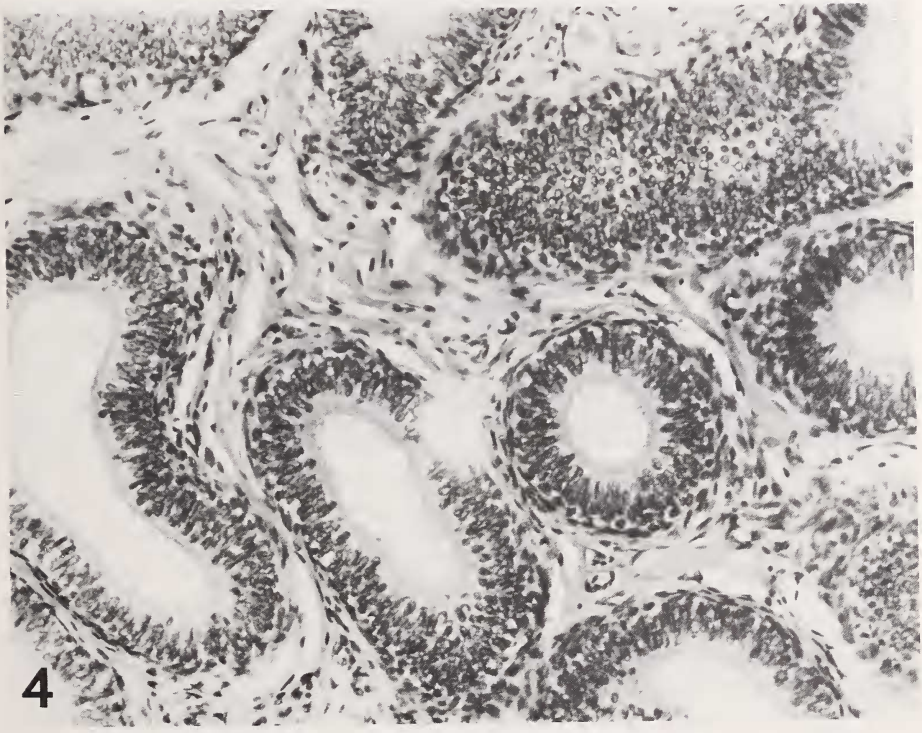

Fig. 4. Epididymis from inactive phase (August). (×200)

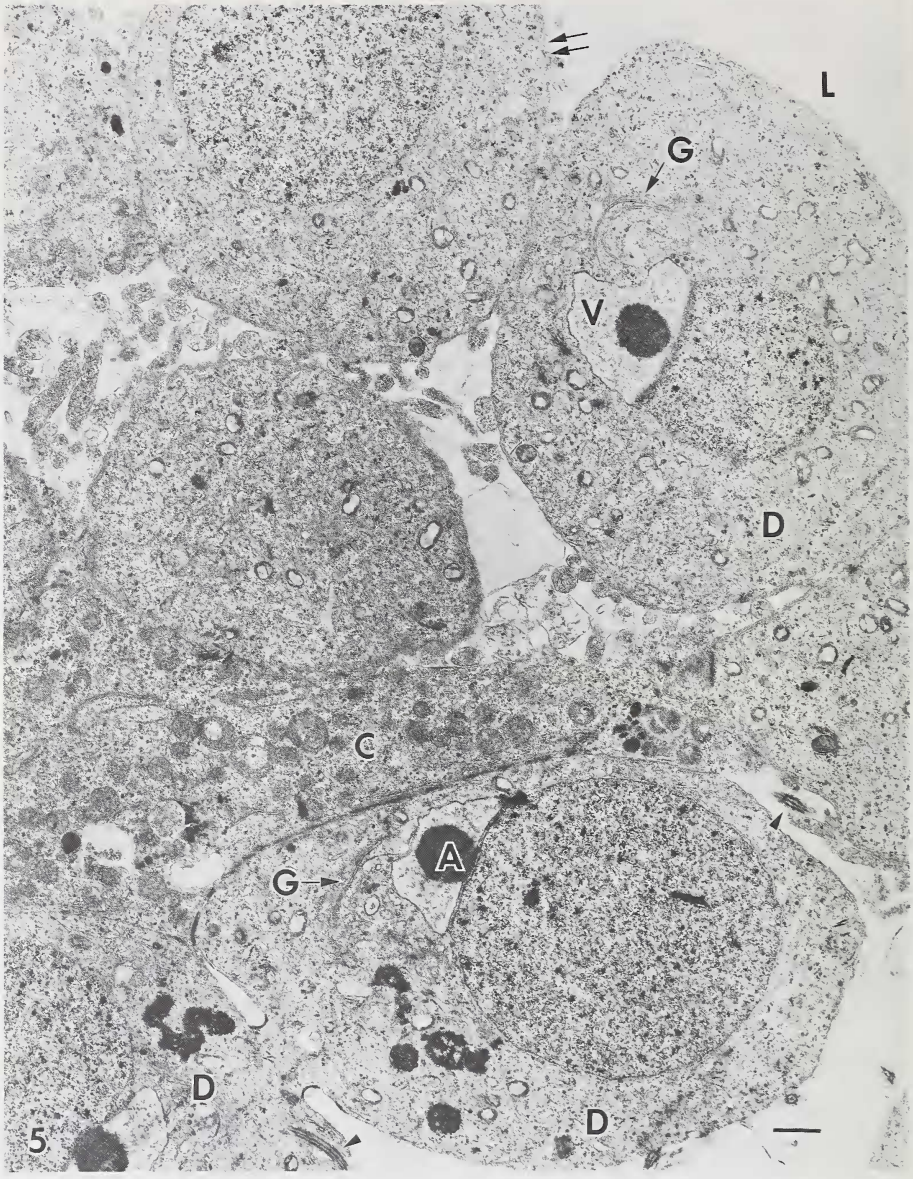

Fig. 5. Spermatids (D) at lumen (L) of active seminiferous tubule (February). Bar = 1 μm. The acrosomal vesicle (V) is at the nuclear membrane in 3 spermatids; it has not developed or is not in the plane of section in another spermatid (double arrow). The acrosomal granule (A) has not redistributed. Sacs of the Golgi complex (G) are in cytoplasm. Tangential sections of the principle piece of spermatozoa (arrowhead) occur. Part of a Sertoli cell (C) is included

Fig. 6. Testes of *Bassariscus astutus. Left:* average size non-breeding season; *right:* average size breeding season

Table 2. **Weights (g) and linear measurements (mm) of testes**

Month	Weight	Length	Width
Jan.	0.96	13.92	10.62
Feb.	1.06	13.42	10.25
Mar.	**1.29**	**14.30**	**12.21**
Apr.	1.09	13.59	10.40
May	1.01	13.34	9.77
June	0.89	12.75	9.08
July	0.71	11.22	7.72
Aug.	0.45	10.51	6.90
Sept.	*0.36*	*10.38*	*6.70*
Oct.	*0.44*	*10.33*	*5.65*
Nov.	0.58	11.54	8.39
Dec.	0.61	12.16	9.16

Bold: maxima; italics: minima.

Measurements are means of left and right testes of a pair. Differences between left and right testis vary from 0 to 0.2 g and 0 to 1.7 mm.

Where more than 1 individual per month was available data were averaged.

Discussion

The physiological capacity to breed is characterized by the mass of the testis and the presence of sperm in the epididymis. The material described in this study clearly indicates an annual developmental cycle of redevelopment and regression of the testes of *Bassariscus astutus*. At age 16 weeks of the young, testes are tiny, ca. 4 mm diameter when descended and palpable, and from then on remain scrotal (Toweill and Toweill 1978). Although

there is no quantitative study, an unknown percentage of young males reach maturity at 10 months of age (Poglayen-Neuwall and Poglayen-Neuwall 1980; Poglayen-Neuwall 1987) and thus are able to mate at or near the peak of the mating season. Of 5 yearling males, held in the senior author's colony, 2 have successfully bred; also 2 wild-caught young captured in March had large testes. Only 20% of yearling male raccoons, according to Wood (1955), possess motile sperm in the Texas post oak region. Among Michigan raccoons many yearling males are capable of reproducing, but females enter estrus about 2 months earlier, and by the time the young males are sexually mature most females are already bred by older males (Stuewer 1943). Active spermatogenesis of *B. astutus* is maximal during the late winter-spring mating season, while it rather abruptly diminishes in June and ceases in July. Our findings show that it takes 1 month plus for spermatogonia to mature to spermatozoa in ringtails, as compared with 20 days in rats (Bloom and Fawcett 1962), and 64 days in humans (Dym 1977).

The number of animals we examined resulted in maximum testis measurements for March, with most active spermatogenesis in April. A broader sample most likely would show a closer correlation between the two. We found the degree of testicular regression in *B. astutus* to be similar to that in *Procyon lotor* (Sanderson and Nalbandov 1973; Wood 1955), and not nearly as stiking as in *Mustela frenata*, whose testes are 1/8 of their maximal size during the peak of their non-breeding season (Wright 1947). Variation of testis size/mass correlated with the season is known in many mammalian species (Amann 1970), e.g. *Martes americana* (Markley and Bassett 1942), *Mustela erminea* and *Vulpes fulva* (Asdell 1964), *Dasypus novemcinctus* (McCusker 1985), many cervids (Amann 1970), several lemurs (Borart et al. 1977; Petter-Rousseaux 1972), *Saimiri sciureus* (Du Mond and Hutchinson 1967), *Macaca mulatta* (Sade 1964) and *Cercopithecus aethiops* on St. Kitts (Conaway and Sade 1969). The latter shows a distinct breeding season (different from that in Kenya), with a testis regression in the non-breeding season, which is much less pronounced than in the aforelisted primates. Sperm were present in the epididymides of all *C. aethiops* examined throughout the year (Conaway and Sade 1969).

The tropical *Bassariscus sumichrasti*, apparently a seasonal breeder, namely from January to May (Gaumer 1917; Hall 1981), does not show palpable testicular regression, as observed on 4 captive adults held by the senior author. No histological study of testes of *B. sumichrasti* has as yet been undertaken. Likewise *Ailurus fulgens*, a seasonal breeder, does not show cyclic variation of the testes' size (J. Gittleman, comm. via g. Conover).

Electroejaculation on an adult *B. astutus* and an adult *B. sumichrasti* (conducted by Dr. B. Durrant and the senior author in early May at the San Diego Zoo's Research Department) produced only minimal volume and very few, non-motile and mutilated spermatozoa. Likewise, electroejaculation on *Procyon lotor* was unsuccessful (Sanderson 1951).

There is a correlation between daily sperm production and testicular weight for continuous breeders and for seasonal breeders during the breeding season (Ortavant, cit. by Amann 1970). On the other hand Martinet's (1966) quantitative, histological studies of *Microtus arvalis* revealed seasonality of the testes weight but there is no seasonal change in the efficiency of sperm production.

Sudden regression of testes during the mating season over a period of only very few weeks and concomitant decrease of active sperm has been noted by us in one newly captured *B. astutus*, perhaps as a direct result of the trauma suffered, and in 2 other ringtails probably as a consequence of constant harassment by a dominant female, which likely caused drastic hormonal changes conducive to the aforementioned reproductive condition. These 3 animals have not been considered in this study.

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Zusammenfassung

Testicularzyklen des Katzenfretts, Bassariscus astutus (Carnivora: Procyonidae)

Mittels Palpierung und morphologisch-histologischen Techniken wurden die Testes von *Bassariscus astutus* im Jahresablauf untersucht. Jahreszeitlicher Wechsel der Spermiogenese wurde mit Gewicht und Größe der Testes verglichen. Es konnte bestätigt werden, daß die Fortpflanzungsperiode in Arizona von Spätwinter bis in den Frühling dauert, und daß die Testes im Sommer und Herbst inaktiv (aspermisch) sind. Sie erreichen ihre kleinsten Maße im Herbst.

References

Amann, R. P. (1970): Sperm production rates. In: The testis. Vol. I. Ed. by A. D. Johnson, W. R. Gomes and N. L. Vandemark. New York: Academix Press. Pp. 433–482.

Asdell, S. A. (1964): Patterns of Mammalian reproduction. Ithaca, New York: University Press.

Basurto-Kuba, V. M.; Heath, E.; Wagner, W. A. (1984): Spermatozoa and testes in the boar: correlative analysis of sperm morphologic features, seminiferous epithelial area, and testes weight. Am. J. Vet. Res. **45**, 1328–1332.

Bloom, W.; Fawcett, D. M. (1962): Textbook of histology. Philadelphia: Saunders. Pp. 550–583.

Borart, M. H.; Cooper, R. W.; Benirschke, K. (1977): Reproductive studies of black and ruffed lemurs, *Lemur m. macaco* and *L. variegatus* ssp. Internat. Zoo Yb. **17**, 77–82.

Conaway, C. H.; Sade, D. S. (1969): Annual testis cycle of the green monkey *(Cercopithecus aethiops)* on St. Kitts, West Indies. J. Mammalogy **50**, 833–835.

Du Mond, F. V.; Hutchinson, T. C. (1967): Squirrel monkey reproduction: the "fatted" male phenomenon and seasonal spermatogenesis. Science **158**, 1067–1070.

Dym, M. (1977): The male reproductive system. In: Histology. Ed. by L. Weiss; R. O. Greep. New York: McGraw-Hill. Pp. 979–1038.

Elcock, L. H.; Schoning, P. (1984): Age related changes in the cat testis and epididymis. Am. J. Vet. Res. **45**, 2380–2384.

Gaumer, G. F. (1917): Monografía de los mamíferos de Yucatán. Secret. de Fomento, Dept. Talleres Gráficos. México.

Grinnell, J.; Dixon, J. S.; Linsdale, J. M. (1937): Fur-bearing mammals of California. Berkeley: University of California Press, Pp. 166–183.

Hall, E. R. (1981): The mammals of North America. New York: John Wiley and Sons. Vol. **2**, 965–966.

Johnson, A. S. (1970): Biology of the raccoon (*Procyon lotor varius* Nelson and Goldman) in Alabama. Agric. Exp. Stat. Auburn Univ. Bull. **402**, 1–148.

Markley, M. H.; Bassett, C. F. (1942): Habits of captive marten. Am. Midl. Nat. **28**, 604–616.

Martinet, L. (1966): Modification de la spermatogènese chez le campagnol des champs *(Microtus arvalis)* en fonction de la durée quotidienne d'éclariment. Ann. Biol. Annale, Biochim., Biophys. **6**, 301.

McCusker, J. S. (1985): Testicular cycles of the common long-nosed armadillo, *Dasypus novemcinctus*, in north-central Texas. In: The evolution and ecology of armadillos, sloths, and vermilinguas. Ed. by G. G. Montgomery. 255–261.

McDowell, E. M.; Trump, F. B. (1976): Histologic fixatives suitable for diagnostic light and electron microscopy. Arch. Path. lab. Med. **100**, 405–414.

Moens, P.; Huigeicholz, A. (1975): The arrangement of sperm cells in the rat seminiferous tubule: an electron microscopic study. J. Cell. Sci. **19**, 487–507.

Mollenhauer, H. H. (1963): Plastic embedding mixture for use in electron microscopy. J. Stain Tech. **39**, 111–114.

Morton, D.; Weisbrode, S. E.; Wyder, W. E.; Maurer, J. K.; Capen, C. C. (1986): Histologic alterations in the testes of laboratory rabbits. Vet. Path. **23**, 214–217.

Ortavant, R. (1958): Le cycle spermatogénetique chez le bélier. D. Sc. thesis, Univ. Paris.

Petter-Rousseaux, A. (1972): Application d'un systeme semestriel de variation de la photoperiode chez *Microcebus murinus* (Miller, 1777). Ann. Biol. Anim. Bioch. Biophys. **12**, 367–375.

Poglayen-Neuwall, I. (1987): Management and breeding of the ringtail or cacomistle, *Bassariscus astutus*, in captivity. Internat. Zoo Yb. **26**, 276–280.
Poglayen-Neuwall, I.; Poglayen-Neuwall, I. (1980): Gestation period and parturition of the ringtail, *Bassariscus astutus* (Lichtenstein, 1830). Z. Säugetierkunde **45**, 73–81.
Sade, D. S. (1964): Seasonal cycle in size of testes in free-ranging *Macaca mulatta*. Folia primat. **2**, 171–180.
Sanderson, G. C. (1951): Breeding habits and a history of the Missouri raccoon population from 1941 to 1948. Trans. Sixteenth N. A. Wildlife Conf. 445–461.
Sanderson, G. C.; Nalbandov, A. V. (1973): The reproductive cycles of the raccoon in Illinois. Ill. Nat. Hist. Surv. Bull. **31**, 25–85.
Stuewer, F. W. (1943): Reproduction of raccoons in Michigan. J. Wildlife Mgt. **7**, 60–73.
Taylor, W. P. (1954): Food habits and notes on the life history of the ring-tailed cat in Texas. J. Mammalogy **33**, 55–63.
Toweill, D. E. (1976): Movements of ringtails in Texas' Edwards Plateau region. Unpubl. MS thesis. College Station: Texas A&M Univ.
Toweill, D. E.; Toweill, D. B. (1978): Growth and development of captive ringtails *(Bassariscus astutus flavus)*. Carnivore 1, 46–53.
Wood, J. E. (1952): Ecology of furbearers in the upland post oak region of eastern Texas. Unpubl. Ph. D. dissert. College Station: Texas A&M. Univ.
— (1955): Notes on reproduction and rate of increase of raccoons in the post oak region of Texas. J. Wildlife Mgt. **19**, 409–410.
Wright, P. L. (1947): The sexual cycle of the male long-tailed weasel *(Mustela frenata)*. J. Mammalogy **28**, 343–352.

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